

CARDIOVASCULAR, PHARMACOLOGY, CHEMISTRY

#307R5

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSION TO THE

TOBACCO INDUSTRY RESEARCH COMMITTEE

COMMITTEE:
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Dr. Bing
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633 THIRD AVENUE
NEW YORK, N. Y. 10017

Activated: 7/1/61
Renewed: 7/1/62

" 7/1/63

" 7/1/64

" 7/1/65

Cf. #137 Activated: 11/15/56

Renewed: 11/15/57

Cf. #221

Activated: 1/1/5
Date: January 24, 1966 Renewed: 7/1/6

Application For Research Grant (RENEWAL)

1. Name of Investigator: Samuel Bellet, M.D., Director, Division of Cardiology
Philadelphia General Hospital

2. Title:
**THE EFFECT OF NICOTINE ON VARIOUS PARAMETERS OF
CARDIOVASCULAR FUNCTION**

3. Institution &
Address:
Philadelphia General Hospital
34th Street & Curie Avenue
Philadelphia, Pennsylvania 19104

4. Project or Subject:

1. Determination of the Urinary Excretion of Nicotine After Smoking and Nicotine in Man and Dog.
2. Effect of Tobacco Substitutes and Special Filters on Catecholamine and Nicotine Excretion.
3. The Effect of Nicotinic Acid on Hyperlipidemia Induced by Tobacco Smoking and Nicotine.
4. Studies of the Effect of Smoking and Nicotine on the Metabolism of Triglycerides and Other Lipids.
5. The Effect of Nicotine on Experimental Atherosclerosis.

5. Detailed Plan of Procedure (Use additional pages if more space is required)
(see continuation sheets 1 and 2)

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6. Budget Plan:

a. Salaries	\$14,519.48
b. Expendable Supplies	2,100.00
c. Other Expenses	600.00
d. Permanent Equipment	
e. Overhead (15% of a, b, c)	
Total	2,882.92
	\$22,102.40

7. Anticipated Duration of Work: One Year

8. Facilities and Staff Available: Pages 3 and 4

9. Additional Requirements: NONE

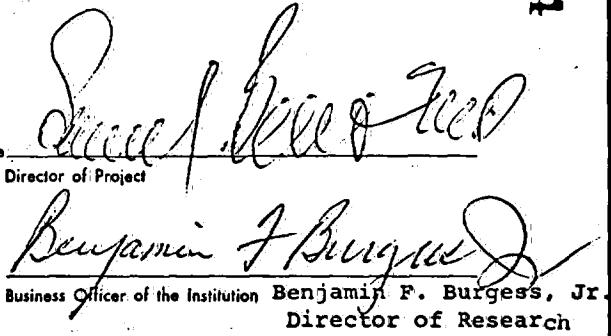
10. Additional Information (Including relation of work to other projects and other sources of support):

NONE

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Signature

Director of Project


Benjamin F. Burgess, Jr.
Business Officer of the Institution
Director of Research

5. Detailed Plan of Procedure:

1. Determination of the urinary excretion of nicotine after smoking and nicotine in man and dog.

Using a modification of the method of McNiven et al., nicotine concentration in urine will be determined by means of gas chromatography. The extraction procedure and column now being used has given us good reproducibility and satisfactory recovery. The following studies will be made:

- (a) A comparison of nicotine excretion with cigarette, cigar, and pipe smoking.
- (b) The effect of inhaling or not inhaling on nicotine excretion.
- (c) The effect of nicotinic acid given before smoking on nicotine excretion (see below).
- (d) The effect of special filters and tobacco substitutes on nicotine excretion compared to regular cigarettes (see below).
- (e) In anesthetized dogs, a comparison of cigarette, cigar, and pipe smoke inhalation and intravenous nicotine on urinary nicotine concentrations.

Preliminary studies have shown significantly greater nicotine excretion with cigarette smoking than cigar or pipe smoking which is reversed when cigars and pipes are inhaled and cigarettes not inhaled.

2. Effect of tobacco substitutes and special filters on catecholamine and nicotine excretion.

In a previous study (see Progress Report) it has been shown that smoking charcoal filter cigarettes and cigarettes containing pipe tobacco have a mobilizing effect on free fatty acids similar to that of standard cigarettes. Cigarettes containing shredded lettuce leaves had no effect on free fatty acids.

In the projected study, this investigation will be extended and the excretion of catecholamines and nicotine with the various cigarette forms will be measured and compared.

3. The effect of nicotinic acid on hyperlipidemia induced by tobacco smoking and nicotine.

There has been considerable interest in recent years in the effect of nicotinic acid (NA) on certain aspects of lipid metabolism. NA reduces plasma levels of FFA and prevents the FFA rise which follows epinephrine and norepinephrine. This interested us in a study of the effect of NA on the lipid effects of smoking and nicotine in human subjects and dogs.

Subjects will smoke 2 cigarettes or an equivalent amount of cigar or pipe tobacco and control curves of the FFA response will be obtained. The experiments will be repeated on a different day with subjects smoking 30 minutes after ingestion of 200 mg of NA. Preliminary results have shown a fall in fasting serum FFA concentration and an inhibiting effect on the rise after smoking. To determine if the FFA effect is secondary to an effect of NA on catecholamine secretion, urinary catecholamines will be measured during 4-hour periods of

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BUDGET PLAN:

a. Salaries

Chief - Laboratory (Part-time)	\$ 3,616.00
(2) Medical Laboratory Technicians (Full time)	10,934.00
Social Security	761.45
Workmen's Compensation	56.17
Fringe Benefits (Full-time only)	320.00
Pension	<u>831.86</u>
	<u>\$16,519.48</u>

b. Consumable Supplies

Dogs, their board, keep, & medical care (Approx. 60 dogs)	\$ 1,500.00
Radioactive Isotopes, Enzymes	200.00
Chemicals, Catheters, ECG & Recording Paper	300.00
Glassware, Instruments, Syringes, Needles, etc.	<u>100.00</u>
	<u>\$ 2,100.00</u>

c. Permanent Equipment

NONE

d. Overhead (15% a,b, & e) \$ 2,882.92

e. Travel - National Meetings, Presentation of Papers \$ 200.00

Repairs

Photography & Reprints \$ 300.00

GRAND TOTAL \$22,102.40

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smoking cigarettes, cigars or pipes with and without NA. To determine the effect of NA on nicotine availability in the body, the nicotine content of the urine collected during 4-hour smoking periods, with and without the ingestion of NA, will be compared.

In dogs under chloralose anesthesia, smoking will be achieved by tracheal intubation. After obtaining control FFA responses to 2 gm of cigarette, cigar, pipe tobacco smoke or equivalent I.V. nicotine, the experiments will be repeated following I.V. administration of 100 mg of NA. The effect of NA on the rise in serum triglycerides which follows smoke inhalation and nicotine (see Progress Report) will also be determined.

4. Studies of the effect of smoking and nicotine on the metabolism of triglycerides and other lipids.

Studies in the human and dog have been initiated to determine the effect of smoking and nicotine on the synthesis and metabolism of tri-, di-, and monoglycerides, cholesterol, cholesterol esters, and phospholipids. Labeled precursor palmitic acid will be administered and the incorporation of the isotopic fatty acid into lipoprotein lipids will be determined by column and thin-layer chromatographic separation and liquid scintillation spectrometry. The effect of smoking and nicotine on the rate and degree of fatty acid incorporation will be studied. These studies should help clarify the mechanism of the rise in serum triglycerides which follows smoking and nicotine in dogs (see Progress Report).

5. The effect of nicotine on experimental atherosclerosis.

Long-term studies are to be done on dogs to determine if the hypercholesterolemia produced by nicotine (see Personal Publications--#1) will have any atherogenic effect when the animals are maintained on a human-type diet (high fat-cholesterol) or are subject to induced experimental atherosclerosis.

Forty dogs will be divided into 4 groups of 10. The first 2 groups will receive a balanced laboratory dog ration. One group will receive daily injection of nicotine in a slow absorption vehicle (see Personal Publication--#1); the other will serve as a control. The second 2 groups will receive the same diet supplemented by 10 gm of cholesterol and 50 gm of butter. One will receive nicotine, the other will be a control group. Half of the dogs will be sacrificed at 3 months and the remainder at 6 months for pathologic study. Serum lipid analyses and electrocardiographic studies will be made at intervals.

Twenty additional dogs will be given a medical thyroidectomy by the administration of radioactive iodine ($I-131$), followed by 2 gm daily doses of propylthiouracil. All of the dogs will receive the cholesterol-butter supplemented diet plus 3 gm of cholic acid daily. Nicotine will be given to 10 dogs and 10 will serve as controls. Studies and sacrifice will be as described above.

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8. Facilities and Staff Available:

(1) General Facilities--

a. A 2,500 bed municipally owned and operated general hospital staffed by the faculties of 5 medical schools, both full-time and part-time. A large percentage of the beds are used for cardiovascular cases. Specialty clinics which are staffed and used by the Division of Cardiology include an adult and a pediatric heart clinic, hypertension clinic, peripheral vascular clinic and diabetic clinic among others. The Division of Cardiology is responsible for all problems relating to the subject of cardiovascular disease for the entire hospital.

b. The Division of Cardiology consists of:

1. A heart station equipped with complete instrumentation for cardiovascular diagnosis: Electrocardiography, Radioelectrocardiography, Phonocardiography, Vectorcardiography, Ballistocardiography, Fluorescopy (Image Intensifier), Cardiac Catheterization, Angiography, Peripheral Vascular Diagnosis (Constant Temperature Room), Library and Reading Room.
2. A biochemical laboratory devoted fully to cardiovascular research (distinct from the hospital clinical laboratory). It is equipped for doing lipid analysis, catecholamine analysis, blood gas analysis, flame photometry, electrophoresis, osmometry, spectrophotometry, colorimetry, chromatography, polarography, etc.
3. An Isotope Laboratory for radioactive tracer studies
4. An Animal Laboratory
5. An Enzyme and Drug Evaluation Laboratory
6. A Pulmonary Function Section

(2) Major Items of Permanent Equipment (Biochemical Laboratory):

- a. Well-type scintillation counter, scaler, probe, rate meter and pulse height analyzer (NRD).
- b. A vibrating reed electrometer (Nuclear-Chicago) with slide chamber, glassware, ionization chambers for measuring weak Beta radioactivity of Tritium, Carbon-14, and Sulphur-35, in solid, liquid and gaseous phase.
- c. Ultracentrifuge
- d. Refrigerator centrifuge
- e. Fraction collector
- f. Column chromatography equipment
- g. Gas chromatography equipment
- h. Paper electrophoresis equipment
- i. Densitometer (Photovolt)
- k. Fluorimeter (Farrand)
- m. Colorimeters (Evelyn-Klett)
- j. Spectrophotometer (Beckman)
- l. Photometer (flame)
- n. Osmometer (Fiske)

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- o. Van Slyke volumetric and manometric apparatus
- p. Scholander respiration air analyzer
- q. pH meters (Beckman Model G and Metrohm)
- r. Polarograph
- s. Respirometer
- t. Dual count rate meter, recorder and scintillation counter combination

(3) Staff--

Director of the Division

Section Chief: Section of General Biochemistry
 Section of Radicisotope Studies
 Section of Adult Hemodynamics
 Section of Pediatric Hemodynamics
 Section of Peripheral Vascular Disease
 Clinical Section
 Section of Drug Biochemical Laboratory
 Section of Animal Research

5 Attending Physicians
1 Electronic Engineer
10 Residents & Fellows in Cardiology
2 Technicians
5 Laboratory Technicians

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